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X-ray Crystallographic Studies of Aspartate Racemase

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Introduction

There exists D-enantiomer of aspartic acid in lactic acid bacteria and several hyperthermophilic archaea, which is biosynthesized from L-enantiomer by aspartate racemase. Aspartate racemase is responsible for the interconversion of L- and D-aspartates. Aspartate racemization is a fundamental proton-transfer reaction, and it is a reciprocal, mirror-symmetrical process. All known amino acid racemases employ a mechanism that involves deprotonation at the α-carbon of the substrate amino acid, followed by reprotonation of the resulting carbanionic intermediate from the reverse side to accompany the inversion of configuration. AARs perform racemizations in two completely different ways, by which they can be further classified into two groups. One group utilizes PLP as a cofactor and the other is PLP-independent. AspR belongs to the PLP-independent group. The "two-base" catalytic mechanism has been proposed for this type of racemase, in which a pair of cysteine residues are utilized as the conjugated catalytic acid and base. We determined the crystal structure of aspartate racemase from *Pyrococcus horikoshii* OT3 at 1.9 Å resolution. Amino acid racemases were not originally thought to exist in archaea, but recently aspartate racemases and D-amino acids were found in some kinds of hyperthermophilic archaea. This enzyme has a favor temperature up to 90°C, and it does not exhibit any activity at room temperature. The subunit of *P. horikoshii* OT3 aspartate racemase consists of 228 amino acid residues with a calculated molecular mass of 25,156 Daltons.

Experimental Results

Aspartate racemase from a hyperthermophilic archaea, *Pyrococcus horikoshii* OT3 has been crystallized with the space group $P2_1$[1]. The intensity data were collected with synchrotron radiation (at BL6A of the Photon Factory and at BL44B2 of SPring-8). The structure was solved by the heavy atom method and refined at 1.9 Å resolution to a crystallographic R factor of 19.4% (R_{free} of 22.2%) (Figure)[2]. This is the first structure of aspartate racemase and also of amino acid racemases from archaea reported to date. The crystal structure revealed that this enzyme forms a stable dimeric structure with a strong three-layered inter-subunit interaction, and that its subunit consists of two structurally homologous α/β domains, each containing a four-stranded parallel β-sheet flanked by six α-helices. Two strictly conserved cysteine residues (Cys82 and Cys194), which have been biochemically shown to act as catalytic acid and base, are located on both sides of a cleft between the two domains. The spatial arrangement of these two cysteines supports the "two-base" mechanism but disproves the previous hypothesis that the active site of aspartate racemase is located at the dimeric interface. The structure also revealed a unique pseudo mirror symmetry in the spatial arrangement of the residues around the active site, which may explain the molecular recognition mechanism of the mirror symmetrical aspartate enantiomers by the non-mirror-symmetrical aspartate racemase.requirements.

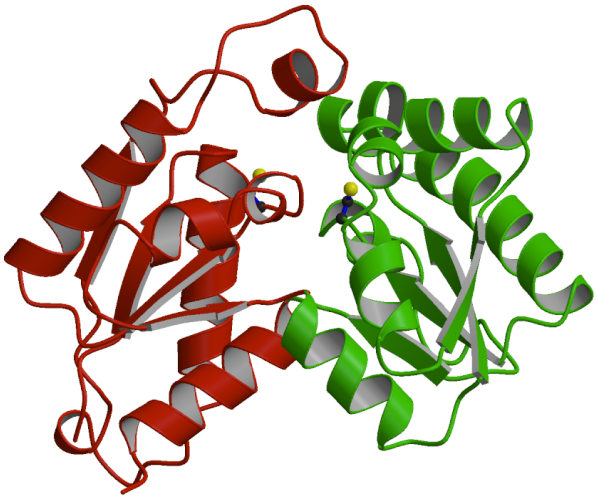

Figure Crystal structure of aspartate racemase from *Pyrococcus horikoshii* OT3 determined at 1.9 Å resolution [2]

References

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2] L. Liu, et al., J. Mol. Biol., 319, 479 (2002).

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